Week 3

June 20, 2016

Notes on NEGEM Presentation

Next slide should explain what rice crispr is
Background slides before defending
In text citations

sgRNA is produced by RNA Pol III
mCherry on separate promotor
Transient transfection in HEK 293T
rtTa tetracyclin repressor

Outreach activities
- ~3 slides
- Say that we have notes to share w W&M and will put on wiki
- Add in touch tomorrow slides, pictures, graphs

Add acknowledgements

Wednesday 3pm run through slides

Draft e-mail to UMASS Zhang, CRISPR Technologies, Team at Harvard/MIT
Start stable integration
Transform and make mini prep
Transfect + add antibiotic
What We Did Today
- Split Cells
- TouchTomorrow Data
- NEGEM Slides
- Transformation for interlab study

Transformation Protocol for interlab study
- Used Plate 2- cell 6F- Bba-R0040 (Cam^r)
  - 10 microliter water and pipetted up and down, put in microcentrifuge tube
  - 1 microliter pRetro on (amp^r)
- Used 50 microliters of dh5a cells and added all of the above to 200 microliters of LB
- Capped tubes and let shake for an hour and a half
- Plated cells and left overnight

<u>GM, JW</u>
Worked on NEGEM PowerPoint

June 21, 2016

What we did today:
- NEGEM Slides
- Interlab study:
  - Checked transformation
  - Made liquid cultures

Liquid Cultures:
- Chose 6 cultures from the pRetro Plates, avoided major satellite cells
- 5mL LB
- 2.5 microliters Ampicillin
- Added cultures to 15 mL conical tube
- Put them in the shaking incubator

Split cell culture (large flask), 1:5

June 22, 2016

What We Did Today:

Finished NEGEM presentation

Looked up people to contact for Integrated Practices

Practiced presentation for NEGEM

• Miniprep

pRetro plasmids: 1006.4 ng/µL

• NEGEM slides

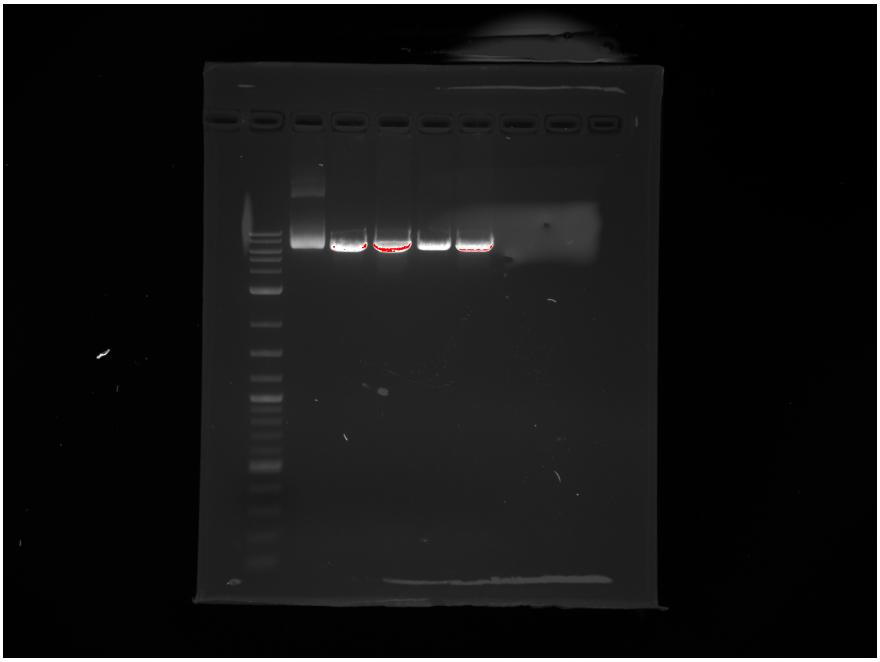
June 23, 2016

What We Did Today:

Ran Gel with Restriction Enzymes

Gel 1: Singles

Lane 1 – Empty
Lane 2 – Ladder
Lane 3 – Neg. Control
Lane 4 – BamH1
Lane 5 – Not1
Lane 6 – Pac 1
Lane 7 – Spe 1 + Cal1

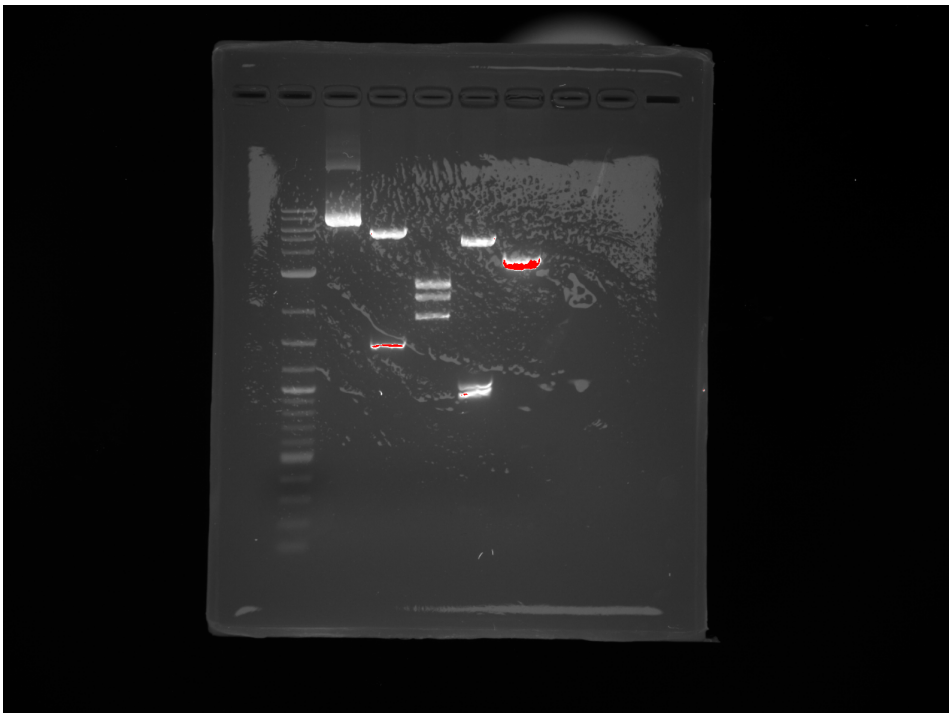


Analysis of Gel 1:

Lane 3: Plasmid Uncut shows band around 6.8kb (size of pRETRO-ON) - Indicates that this is the correct plasmid
Lane 4: BamH1 had 1 cut site -> resulting in one fragment (6.8kb)
Lane 5: Not1 had 1 cut site -> resulting in one fragment (6.8kb)
Lane 6: Pac1 had 1 cut site -> resulting in one fragment (6.8kb)
Lane 7: Spe1 had 1 cut site and Cal1 had no cut sites -> resulting in one fragment (6.8kb)

Gel 2: Multiple

Lane 1 – Empty
Lane 2 – Ladder
Lane 3 – Neg. Control
Lane 4 – EcoR1 + BamH1
Lane 5 – xBa1 + HindIII
Lane 6 – EcoRI +Pst1
Lane 7 – xHo1 + Spe1



Analysis of Gel 2:

Lane 3: Plasmid Uncut shows band around 6.8kb (size of pRETRO-ON) - Indicates that this is the correct plasmid

Lane 4: EcoR1 has a cut site between nucleotides 1813 and 1814; BamH1 has a cut site between nucleotides 461 and 460 -> 2 fragments (one at nt 1352 and one at nt 5482). The bands in this gel are around the 1.5kb mark and in between the 5kb and 6kb marks.

Lane 5: xBa1 has 3 cut sites at nts 1444, 3675, and 6284; HindIII has 1 cut site at nt 1351 -> 4 fragments (93b, 2231b, 2609b, and 1901b). The bands in the gel around 2kb showing the three larger fragments. The fragment that is 93b is too small to see.

Lane 6: EcoR1 has 1 cut site at nt 1814; Pst1 has 3 cut sites at nt 2813, 2989, and 3861 -> 4 fragments (999b, 176b, 872b, 4787b). The band in the gel between 4 and 5 kb show one fragment. The bands in the gel around 1kb show two fragments. The 176b fragment is too small to see on the gel.

Lane 7: xHo1 has 1 cut site at nt 6835; Spe1 has 1 cut site at nt 3242 -> 2 fragments (3593b and 3241b). The two bands around the 3kb mark show both of these fragments.

Restriction Digest of Plasmids

- BamHI+EcoRI
- PacI
- BamHI
- NotI

15-16uL water
2uL SmartCut
1uq plasmid
1uL enzyme

Heat incubate 1-2 hours
Run gel

Big flask - bacterial contamination
Small - fungal
Threw out all media

Streaked humanized dcas9 on amp and 2x amp plates

• Digest: ECoRI, PstI (+ pRetro plasmid)
    Total = 20 µL
    - 2 µL CutSmart buffer
    - 1 µg pRetro
    - 1 µL each enzyme
• Streaked plates: pgRNA-humanized (1X & 2X Amp plates, 1 each)